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The Shiverer Mutation Affects the Persistence of Theiler's Virus in the Central Nervous System

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Theiler's virus persists in the white matter of the spinal cord of genetically susceptible mice and causes primary demyelination. The virus persists in macrophages/microglial cells, but also in oligodendrocytes, the myelin-forming cells. Susceptibility/resistance to this chronic infection has been mapped to several loci including one tentatively located in the telomeric region of chromosome 18, close to the myelin basic protein locus (*Mbp* locus). To determine if the MBP gene influences viral persistence, we inoculated C3H mice bearing the shiverer mutation, a 20-kb deletion in the gene. Whereas control C3H mice were of intermediate susceptibility, C3H mice heterozygous for the mutation were very susceptible, and those homozygous for the mutation were completely resistant. This resistance was not immune mediated. Furthermore, C3H/101H mice homozygous for a point mutation in the gene coding for the proteolipid protein of myelin, the rumpshaker mutation, were resistant. These results strongly support the view that oligodendrocytes are a necessary viral target for the establishment of a persistent infection by Theiler's virus.

The Daniel (DA) strain of Theiler's murine encephalomyelitis virus (TMEV), a picornavirus, is responsible for a biphasic neurological disease of mice (12, 18). The first phase, or early disease, is an acute encephalomyelitis that takes place during the first 2 weeks following intracerebral inoculation. At this stage, the virus replicates mostly in neurons of the brain and spinal cord. The virus then disappears from the gray matter and migrates to the white matter of the spinal cord (4). During the ensuing late disease, it persists in macrophages/microglial cells, oligodendrocytes, and possibly astrocytes for the lifetime of the animal (1, 10, 19). This persistent infection is associated with meningitis, mononuclear inflammation of the white matter parenchyma, and primary demyelination (11). Because of its chronicity and the histological features of the lesions, this late demyelinating disease is studied as a model for multiple sclerosis.

All inbred strains of mice are susceptible to the early gray matter infection. However, inbred strains show various degrees of susceptibility to the persistent infection and the accompanying late disease (23–25). Susceptibility to the persistent infection has been linked to the *H-2D* region of the major histocompatibility complex. The *b* haplotype is associated with resistance. The *d*, *k*, and *s* haplotypes are associated with intermediate susceptibility, and the *q* haplotype is associated with full susceptibility (6). The *H-2D*^{b} gene itself is responsible for resistance since susceptible *H-2*^{q} FVB mice transgenic for the *H-2D*^{b} gene become resistant (2). However, non-*H-2* loci also control viral persistence since the SJL/J mouse is more susceptible than the B10.S mouse, although both have the same *H-2*^{s} haplotype. We mapped one gene responsible for the susceptibility of the SJL/J mouse close to the *Ifg* locus on chromosome 10, using an (SJL/J × B10.S)F_1 × B10.S backcross (5). Another gene was tentatively mapped to the telomeric part of chromosome 18, close to the MBP gene, which codes for the myelin basic protein (5). The present work was undertaken to test if the MBP gene could indeed affect the persistence of TMEV.

Myelin, which in the central nervous system (CNS) is an extension of the oligodendrocyte, is a multilamellar membrane that sheathes axons and facilitates the saltatory conduction of nerve impulses. MBP and proteolipid protein (PLP) are the major proteins of CNS myelin. MBP is a family of peripheral membrane proteins which are most probably involved in interactions between the intracellular leaflets of the myelin membrane (30). The MBP gene is part of a large gene complex called Golli-mbp (gene expressed in the oligodendrocyte lineage) (8). This complex consists of 11 exons spread over 105 kb. Three transcription start sites have been described. The major one gives rise to at least eight transcripts, through alternative splicing of the last seven exons (exons 5B to 11). Another transcription start site, located 32 kb upstream of the major one, gives rise to an additional transcript, called M41 or MBP2, that uses two additional exons (exons 4 and 5A) (16). These transcripts are expressed only in oligodendrocytes. A third transcription start site is located 73 kb upstream of the major one. It gives rise to several transcripts, some of which are expressed not only in oligodendrocytes but also in neurons and even outside the CNS in the thymus, spleen, bone marrow, and peritoneal macrophages, as well as in T-cell and B-cell lines (7, 14, 35).

The shiverer (*shi*) mutation is a 20-kb deletion of the last five exons of the MBP gene (26). This deletion affects all MBP transcripts except one which consists of the first three exons and uses the upstream promoter. Homozygous shiverer mice have normal or even elevated numbers of oligodendrocytes, but the amount of CNS myelin is grossly reduced and its ultrastructure is abnormal (28, 30, 32). The mutation causes violent shivering that increases in severity with age. Heterozygous shiverer mice have myelin which is structurally normal, although its MBP content is reduced by approximately 50%. They do not show clinical signs.

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PLP, the other major myelin protein, is a transmembrane protein most likely involved in adhesion of the extracellular leaflets of the myelin membrane (3). The PLP gene is located on the X chromosome and consists of seven exons (21, 22). A point mutation in this gene, the rumpshaker (*rsh*) mutation, has been described in the mouse. Homozygous mutants have an increased number of oligodendrocytes but a severe CNS hypomyelination and tremors (15, 33).

In the present article, we show that, whereas the C3H +/+ mice are of intermediate susceptibility to persistent infection by TMEV, the C3H +/*shi* mice are highly susceptible and the C3H *shi*/*shi* mice are totally resistant. This resistance is not immune mediated. We also show that the *rsh*/*rsh* mutant is resistant. These results make the MBP gene a strong candidate for the control of TMEV's persistence. They support the notion that the infection of oligodendrocytes is necessary for the persistence of TMEV in the CNS.

MATERIALS AND METHODS

Mice. Two male and two female C3HHeB/FeJ +/*shi* mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). The C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice used for this study were derived from this initial set of mice at the Pasteur Institute animal facility. C3H *shi*/*shi* mice were identified on the basis of clinical signs and PCR amplification of the Mbp dinucleotide repeat microsatellite (20). This marker is polymorphic between the C3H +/+ and the C3H *shi*/*shi* mice because the shiverer mutation occurred in an outbred mouse and was subsequently transferred on the C3HHeB/FeJ background. C3H +/+ and C3H +/*shi* mice could not be distinguished with the Mbp marker because, for unknown reasons, only the + allele is amplified from C3H +/*shi* DNA. Therefore, to differentiate the C3H +/*shi* mice from the C3H +/+ mice, we used a couple of primers (5′-GAGAGATGCGTGCTGACAGG-3′ and 5′-GTGTGTGAACA CCTGCCTGC-3′) located on each side of the shiverer deletion. PCR with these primers amplifies a 449-bp product only from C3H +/*shi* and C3H *shi*/*shi* DNAs. Amplification was performed as described previously (27) with an annealing temperature of 55°C.

The *rsh*/*rsh* mice were kindly provided by I. Griffiths (Medical Research Council, Glasgow, Great Britain) and F. La Chapelle (La Salpêtrière, Paris, France). Their genetic background is a hybrid between the C3H and the 101/H inbred mice.

Viral inoculation. The DA and GDVII strains of TMEV were plaque purified three times on BHK-21 cells. Three- to four-week-old anesthetized mice were inoculated intracranially with either 10^5 or 10^6 PFU of TMEV strain DA or 10^4 PFU of the GDVII strain in 40 μl of phosphate-buffered saline (PBS).

Irradiation procedure. Mice were irradiated at 4.5 Gy and inoculated 1 day later as described above. Nonirradiated control mice were inoculated at the same time.

Preparation of total RNA and dot blot analysis. Anesthetized mice were perfused through the left ventricle with 20 ml of PBS, and their brains and spinal cords were immediately removed. Total RNA was extracted by the procedure of Chomczynski and Sacchi (9) and quantified by spectrophotometry. For each mouse, four serial fivefold dilutions of total RNA from the brain or spinal cord were dot blotted, starting with 10 μg per dot, on Hybond C-extra filters (Amersham) and hybridized with a ^{32}P-labeled cDNA probe specific for either the 5′ extremity of the TMEV genome or the β-actin mRNA. For each sample, the highest dilution which gave a positive hybridization signal after a standard exposure time was used as a measure of viral RNA content. This hybridization score varied between 0 and 4. To compare the results obtained in different hybridization experiments, the RNAs of three mice from each experiment were hybridized again together on the same filter. The hybridization scores obtained, when slightly different from the original ones, were used to normalize all the data presented in Table 1. A mean and a standard error of the mean were calculated for each group of mice. The results between two groups were compared by the nonparametric test of Mann and Whitney. The Student *t* test gave similar results. The assay has been described in detail elsewhere (6).

Histological analysis. The histological techniques have been described in detail elsewhere (1). Anesthetized mice were perfused through the left ventricle with 20 ml of PBS followed by 20 ml of 4% paraformaldehyde dissolved in PBS. Brain and spinal cord were dissected, cut into tissue blocks, and embedded in paraffin. Coronal sections of brain and longitudinal sections of the entire spinal cord (7 μm thick) were examined for histopathology and the presence of viral antigens. TMEV capsid antigens were detected with a primary rabbit anticapsid serum, a secondary biotinylated goat anti-rabbit immunoglobulin, and the ABC peroxidase detection system (Vector Laboratories). The sections were counterstained with hematoxylin.

Flow cytometry analysis. Dissected spleen, thymus, and mesenteric lymph nodes were dissociated in Dulbecco modified Eagle medium containing 3% fetal calf serum (FCS), centrifuged for 5 min at 1,500 rpm, and resuspended in an appropriate volume of RPMI medium containing 3% FCS. Resident peritoneal cells were collected as described by Fortier and Falk (13). Peritoneal content was harvested in Dulbecco modified Eagle medium containing 3% FCS and centrifuged for 5 min at 1,500 rpm, and the pellet was resuspended in an appropriate volume of RPMI medium containing 3% FCS. Thymocytes, splenocytes, mesenteric and cervical lymph node cells, and peritoneal cells from groups of three mice were analyzed. Flow-cytometric analysis (FACScan; Becton Dickinson) was performed on these cells by using phycoerythrin-conjugated CD45R/B220 monoclonal antibody (MAb) (Pharmingen), fluorescein isothiocyanate (FITC)-conjugated Mac3 MAb (Pharmingen), FITC-conjugated CD3 MAb (Pharmingen), phycoerythrin-conjugated L3/T4 MAb (Tebu S.A.), FITC-conjugated Ly-2 MAb (Tebu S.A.), and a rabbit polyclonal antibody against the F4/80 marker (34) followed by an FITC-conjugated anti-rabbit immunoglobulin (Serva).

RESULTS

We examined the effects of the shiverer mutation on both the early encephalomyelitis and the ensuing spinal cord infection by inoculating C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice with the DA strain of TMEV. Brain and spinal cord were examined at days 6, 20, and 45 postinoculation (p.i.). Viral RNA was quantitated in these tissues by dot blot hybridization. Histopathology and the presence of viral antigens in tissue sections were also examined.

Effect of the shiverer mutation on early encephalomyelitis. Viral RNA was present in the brain in all the mice examined 6 days p.i., regardless of their genotype. The levels of viral RNA were similar in the three groups (Fig. 1A; Table 1). Histological studies were performed on the brains and spinal cords of five mice from each genetic group. The findings were similar for the three groups. Infected cells, with the appearance of neurons, were present in the gray matter of brain and, in approximately half of the animals, in the gray matter of spinal cord. Inflammation of the gray matter was found in association with infection. Mild meningitis was present in some animals. Therefore, the early gray matter encephalomyelitis due to TMEV is not affected by the shiverer mutation.

This was confirmed by taking advantage of the GDVII strain of TMEV, a strain which causes an acute, fatal, early gray matter encephalomyelitis in wild-type mice. Mortality rates were compared for groups of 12 C3H +/+, 5 C3H +/*shi*, and 8 C3H *shi*/*shi* mice. No significant differences were found (data not shown).

Effect of the shiverer mutation on late persistent infection. Twenty days p.i., striking phenotypic differences were observed between the three groups of mice. As shown in Fig. 1B and Table 1, the spinal cords of the C3H +/+ and C3H +/*shi* mice, six and seven animals respectively, contained significant amounts of viral RNA. In contrast, no viral RNA was detected in the spinal cord in the C3H *shi*/*shi* mice (six animals). Histopathological studies were performed on the brains and spinal cords of 7 C3H +/+, 11 C3H +/*shi*, and 10 C3H *shi*/*shi* mice sacrificed at the same time p.i. A few infected cells were observed in the gray matter of the brain in the C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice. In the spinal cord, numerous infected cells were found in the white matter in all the C3H +/*shi* mice examined. They were associated with perivascular cuffs and diffuse parenchymal infiltration by mononuclear cells. Similar observations were made for the spinal cords of the C3H +/+ mice, although there was a greater variation in the intensity of the lesions from mouse to mouse. In contrast, no evidence for infection was found in the spinal cords of the C3H *shi*/*shi* mice, although mild inflammation was observed in some of them (Fig. 2).

The viral-RNA content of the spinal cord was also examined 45 days p.i. As shown in Table 1 and Fig. 1C, small amounts of viral RNA were observed in the 19 C3H +/+ mice examined, whereas in 9 C3H +/*shi* mice the amounts of viral RNA were similar to those found 20 days p.i. No viral RNA could be detected in the spinal cords of the 19 C3H *shi*/*shi* mice examined. Histological studies were performed at the same time p.i.

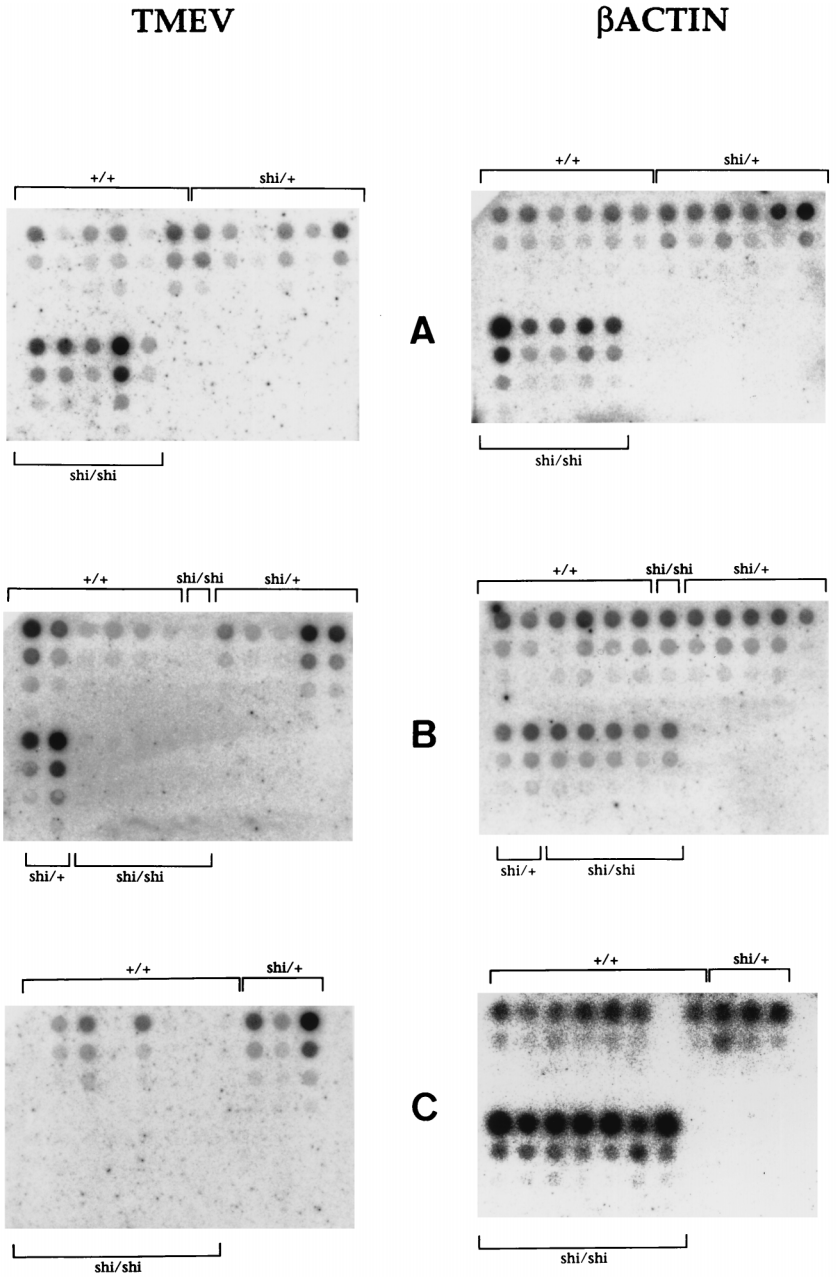

FIG. 1. Amount of viral RNA in the CNS in C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice. The amount of viral RNA was measured with a dot blot hybridization assay, as described in detail in Materials and Methods. Serial fivefold dilutions of total RNA extracted from brain or spinal cord (10, 0.2, 0.04, and 0.008 μg of RNA per dot) were blotted on filters in duplicate and hybridized with a ^{32}P-labeled cDNA probe specific for either TMEV RNA or β-actin mRNA. (A) RNAs from the brains of mice sacrificed 6 days p.i.; (B) RNAs from the spinal cords of mice sacrificed 20 days p.i.; (C) RNAs from the spinal cords of mice sacrificed 45 days p.i.

on longitudinal sections of the entire spinal cords of 4 C3H +/+, 9 C3H +/*shi*, and 10 C3H *shi*/*shi* mice. No infected cells or inflammation was observed in the C3H *shi*/*shi* mice. Only occasional infected cells were found in the case of the C3H +/+ mice. In contrast, numerous infected cells were present in the white matter of spinal cord in eight of the nine C3H +/*shi* mice examined. Infected areas showed inflammation, particularly perivascular cuffs of mononuclear cells.

To sum up, there was good agreement between the data obtained by measuring viral RNA and by detecting viral antigens in tissue sections. The experiments confirmed that C3H +/+ mice are of intermediate susceptibility to persistent infection by TMEV. C3H mice heterozygous for the shiverer mutation were fully susceptible. However, C3H mice homozygous for the shiverer mutation were completely resistant.

The effect of the shiverer mutation on persistent infection is not mediated by the immune response. We examined the possibility that the shiverer mutation, by affecting the immune responses of the C3H mice, increased the efficacy of viral clearance. In the first type of experiment, we compared the lymphocyte populations of the C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice before and after inoculation with TMEV. Lymphocytes were prepared from the thymus, spleen, and mesenteric lymph nodes of uninfected mice and analyzed by flow

TABLE 1. Amount of viral RNA in the CNS measured by a dot blot hybridization assay[a]

Mice and day p.i.	Inoculum (PFU)	Irradiation	Amt of RNA[b] (*n*)
+/+			
6	10^5	No	1.7 ± 0.2 (6)
20	10^5	No	1.5 ± 0.4 (6)
45	10^5	No	0.8 ± 0.2 (19)
20	10^6	No	0.7 ± 0.4 (5)
45	10^5	Yes	2.7 ± 0.5 (8)
+/*shi*			
6	10^5	No	1.6 ± 0.2 (6)
20	10^5	No	2.3 ± 0.4 (7)
45	10^5	No	2.4 ± 0.2 (9)[c]
20	10^6	No	1.4 ± 0.6 (5)
shi/*shi*			
6	10^5	No	1.9 ± 0.3 (5)
20	10^5	No	0.0 ± 0.0 (6)[d]
45	10^5	No	0.0 ± 0.0 (19)[c]
20	10^6	No	0.0 ± 0.0 (5)
45	10^5	Yes	0.0 ± 0.0 (3)[e]
rsh/*rsh*			
20	10^5	No	0.0 ± 0.0 (6)[d]
20	10^6	No	0.0 ± 0.0 (3)
45	10^5	Yes	0.0 ± 0.0 (6)[e]

[a] The RNA was extracted from the brains of mice sacrificed 6 days p.i. For all other times, the RNA was extracted from the spinal cord.
[b] On a scale from 0 to 4 (see Materials and Methods). The data are means ± standard errors of the means.
[c] Statistically different from the RNA content for C3H +/+ mice sacrificed 45 days p.i. ($P < 0.01$).
[d] Statistically different from the RNA content for C3H +/+ mice sacrificed 20 days p.i. ($P < 0.01$).
[e] Statistically different from the RNA content for irradiated C3H +/+ mice sacrificed 45 days p.i. ($P < 0.01$).

cytometry. The total number of T lymphocytes and the numbers of $CD4^+$ and $CD8^+$ T lymphocytes were not significantly different between the three groups of mice. Likewise, there were no significant differences between the numbers of spleen and lymph node B lymphocytes or the numbers of peritoneal macrophages (data not shown).

We then compared the B- and T-lymphocyte populations in the cervical lymph nodes of mice of each genotype, before and 6 days after intracranial inoculation with TMEV. In these experiments, the lymph nodes of three mice in each category were pooled before analysis. Interestingly, the numbers of B lymphocytes increased significantly after inoculation, whereas the numbers of $CD4^+$ T lymphocytes decreased. The numbers of $CD8^+$ T lymphocytes remained unchanged. However, these variations were the same regardless of the genotype of the mouse (data not shown).

As a rule, resistance of certain inbred strains of mice to TMEV persistent infection, which is in great part immune mediated, can be abrogated by increasing the dose of virus inoculated or by gamma irradiation. Groups of C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice were inoculated with 10^6 PFU of TMEV, instead of 10^5 PFU, and examined 20 days later. Table 1 shows that the C3H *shi*/*shi* mice were resistant even under these conditions. Whole-body irradiation was performed on C3H +/+ and C3H *shi*/*shi* mice 1 day prior to intracranial inoculation with 10^5 PFU of TMEV. The course of the infection was examined 20 and 45 days p.i. either by measuring viral RNA in the CNS with the dot blot assay, by performing immunocytochemistry on tissue sections, or both. Twenty days p.i., the CNSs of both groups of mice were infected to similar levels, as shown by immunocytochemistry. Virus was detected mainly in the gray matter of brain but also in the gray matter of spinal cord (data not shown). In nonirradiated control mice examined in parallel, the virus had been cleared from the CNSs of the C3H *shi*/*shi* mice, whereas it was still present in those of the C3H +/+ mice. Forty-five days p.i., viral RNA was present in large amounts in the spinal cords of irradiated C3H +/+ mice, as shown by dot blot hybridization (Table 1). The amount of viral RNA was higher than in control nonirradiated

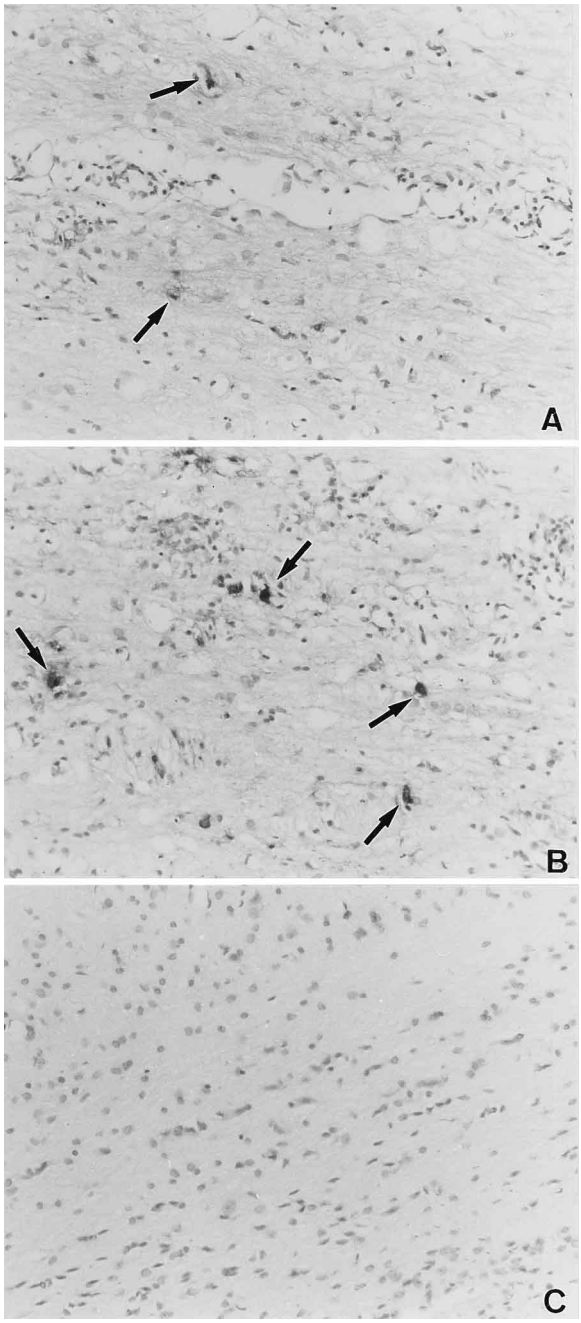

FIG. 2. Histological findings in the spinal cords of mice of the C3H +/+ (A), C3H +/*shi* (B), and C3H *shi*/*shi* (C) genotypes. The mice were sacrificed 20 days p.i. Longitudinal sections of spinal cord were prepared, reacted with an anti-TMEV serum to detect viral antigens by immunocytochemistry (arrows), and counterstained with hematoxylin.

C3H +/+ mice. The virus was located mainly in the white matter of the spinal cord, as shown by immunocytochemistry (not shown). In contrast, no viral RNA (Table 1) and no viral antigens (not shown) were found in the spinal cords of irradiated C3H *shi*/*shi* mice.

In conclusion, the shiverer mutation did not affect the immunological parameters which we examined. Shiverer mice were still resistant after inoculation with 10^6 PFU of TMEV or after gamma irradiation, a phenotype which has not been observed before. Therefore, the mutation confers a new resistant phenotype which is, most likely, not immune mediated.

The rumpshaker mice are also resistant to TMEV persistent infection. We considered the possibility that the resistance of the shiverer mouse was due to an inherent property of the oligodendrocytes of this mutant. We tested this hypothesis with the rumpshaker mutation, another mutation affecting myelin. Rumpshaker mice have a C3H–101/H hybrid background and bear the *H-2*^{k} haplotype which confers intermediate susceptibility. They were inoculated with 10^5 PFU of TMEV and sacrificed at days 6 and 20 p.i. Viral infection was studied with a combination of the dot blot assay and immunocytochemistry, as described above for the shiverer mouse.

Six days p.i., histological studies did not show any difference between the brains or spinal cords of the *rsh*/*rsh* mice and those of the C3H +/+, C3H +/*shi*, and C3H *shi*/*shi* mice. In particular, the amounts of viral antigens and the distributions of infected cells were very similar (data not shown). Therefore, the early disease was not influenced by the mutation. On the other hand, no viral RNA was observed in the spinal cords of the six *rsh*/*rsh* mice 20 days p.i. (Table 1). We examined if this resistance could be overcome by increasing the dose of virus inoculated or by gamma irradiation. As shown in Table 1, *rsh*/*rsh* mice inoculated with 10^6 PFU of TMEV had no viral RNA in the spinal cord 20 days p.i., and gamma irradiation did not increase the susceptibility of these mice. Thus, the homozygous rumpshaker mutation, like the shiverer mutation, confers resistance on mice with a susceptible *H-2*^{k} background.

DISCUSSION

We have shown previously that a locus near *Mbp* might contribute to the susceptibility of SJL/J mice to persistent infection of CNS by TMEV (5). Since MBP is expressed by two of the virus target cells, the oligodendrocyte and the macrophage/microglial cell (7, 14, 35), we decided to investigate the possibility that the MBP gene might control the persistence of TMEV. To examine this question, we used the shiverer mutation, a large deletion which inactivates all the "classical" transcripts of this gene and all but one of the "new" transcripts (17). This mutation has been transferred on the C3H background, a background associated with intermediate susceptibility to the persistent infection (6). Our results showed a strong effect of the shiverer mutation on susceptibility since (i) in contrast to C3H +/+ mice, C3H *shi*/*shi* mice cleared the infection by 20 days p.i., and (ii) C3H +/*shi* mice had considerably more viral RNA in the CNS than C3H +/+ mice, particularly at 45 days p.i.

In our previous studies, susceptibility was defined by the amount of viral RNA present in the spinal cord 45 days after inoculation with 10^4 PFU of the DA strain of TMEV (2, 5, 6). In the present study mice were inoculated with 10^5 and, in some cases, 10^6 PFU of TMEV. This was because the control wild-type mice were of intermediate susceptibility. Therefore, using a higher inoculum magnified the effects of the shiverer and rumpshaker mutations. The dot blot assay which we used is simple to perform and gives more reproducible results than an infectivity assay (5, 6). The results are in complete agreement with those obtained by detecting viral RNA by in situ hybridization or viral antigens by immunocytochemistry.

The background of the rumpshaker mutant is a hybrid between the C3H and 101/H strains. Unfortunately, the +/*rsh* mouse, from which a control +/+ mouse could have been bred, was not available. However, since the C3H and 101/H strains, which bear the *H-2*^{k} haplotype, are of intermediate susceptibility (6; also, data not shown), it is most likely that the +/+ strain would be also of intermediate susceptibility. Furthermore, the *rsh*/*rsh* mice were resistant after inoculation with 10^6 PFU or after gamma irradiation, a phenotype which has not been observed in wild-type inbred mice. Therefore, it is most likely that the phenotype of the *rsh*/*rsh* mouse, which is similar to that of the *shi*/*shi* mouse, is due to the PLP mutation.

Shiverer is a spontaneous mutation which occurred in an outbred mouse and was later transferred on the C3H background. We cannot formally exclude the possibility, although it seems very unlikely, that the phenotypes of the C3H +/*shi* and C3H *shi*/*shi* mice might be due to a gene transferred together with the MBP gene during backcrossing, but distinct from it. The fact that rumpshaker, a mutation in another myelin gene, also confers resistance to persistent infection, speaks much in favor of a role of the MBP and PLP genes themselves in the phenotypes described in this article.

The MBP gene is a complex gene, with 11 exons, two main transcription start sites and several alternative patterns of mRNA splicing. Several mRNAs are expressed exclusively in the oligodendrocyte, but others are expressed also in nonneural tissues such as thymus, spleen, bone marrow, and peritoneal macrophages, as well as several B- and T-cell lines. It was therefore conceivable that the MBP gene might affect the persistence of TMEV infection by modulating the immune response to this infection. Our results (Table 1) strongly suggest that this is not the case, although we cannot formally exclude immune resistance mediated exclusively by macrophages, since these cells are not eliminated by gamma irradiation. Gamma irradiation exacerbated and lengthened the early gray matter infection of both the C3H +/+ and the C3H *shi*/*shi* mice. On the other hand, whereas irradiated C3H +/+ mice had exacerbated late white matter disease, presumably because of immunosuppression, the C3H *shi*/*shi* mice were resistant. This difference of behavior emphasizes that (i) the shiverer mutation affects not the early neuronal phase of the infection, but only the late white matter phase, (ii) and the resistance of the C3H *shi*/*shi* mice is most probably not immune mediated.

The number of oligodendrocytes is normal, or even increased, in shiverer and rumpshaker mutant mice, although the myelin is grossly deficient (15, 28, 30, 32). Therefore, the resistance of these mice to TMEV persistent infection is not due to the absence of oligodendrocytes, one of the viral target cells. It is possible that MBP and PLP are among the cellular proteins which interact with viral products to ensure, e.g., efficient replication. Mutations in either gene could make the oligodendrocyte nonpermissive. MBP seems to be expressed also in macrophages (35), another type of target cell for the virus. Therefore, the shiverer mutation could affect viral replication in this cell type as well. Since a common consequence of both the shiverer and the rumpshaker mutation is a great reduction in the amount of myelin, and structural abnormalities in the little that is present, it is possible that resistance to the infection is linked to this structural and functional defect, e.g., the virus may have to replicate in, or traffic through, the myelin sheath in order to escape detection by the immune system or in order to reach another target cell such as the microglial cell/macrophage which is the main reservoir of virus during the

persistent phase of the infection (10, 29). Interestingly, TMEV antigens have been described in the paranodal terminal loops and cytoplasmic channels of myelin in persistently infected mice (31). Alternatively, the massive breakdown of myelin that follows infection of wild-type mice might be needed to recruit and activate macrophages in the CNS, thereby providing the virus with a critical target cell. The amount of viral RNA decreases with time in the spinal cord in C3H +/+ mice, whereas it remains high in the C3H +/*shi* mice (Table 1). The increased susceptibility of the C3H +/*shi* mice is difficult to interpret since these mice have normal-appearing myelin. However, the amount of MBP in their myelin is approximately half that in wild-type mice, again pointing to a role for MBP in the control of viral replication. This increased susceptibility is also remarkable in that it could not be overcome by a normally dominant *H-2*^{b} restricted immune response. Indeed, (C57BL/6 × C3H +/*shi*)F_1 mice were still fully susceptible, whereas, as expected, (C57BL/6 × C3H +/+)F_1 mice were resistant (data not shown). Regardless of the mechanisms underlying the phenotypes described in this work, our results indicate that oligodendrocytes play a critical role in TMEV persistent infection.

Finally, we would like to emphasize two points. First, the homozygous shiverer and rumpshaker mice are the most resistant mice described so far in this infection, and the heterozygous shiverer mouse is among the most susceptible. Second, MBP, which is the antigen responsible for experimental autoimmune encephalomyelitis, a classical experimental autoimmune disease, has been implicated in susceptibility to multiple sclerosis (34). Our results show that autoimmunity is not the only way in which this protein can be involved in susceptibility to a demyelinating disease.

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